Kidney International, Vol. 49 (1996), pp. 1199–1206

LABORATORY INVESTIGATION

Role of adenosine in the renal responses to contrast medium

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Role of adenosine in the renal responses to contrast medium. Despite the development of non-ionic radiographic contrast media (CM), CM-induced nephropathy is a clinically important problem in patients with pre-existing renal insufficiency. We examined the effects of non-ionic CM (iohexol) on renal function in conscious dogs with and without renal insufficiency, and evaluated the effects of a non-selective (theophylline), an A1 selective (KW-3902), and an A2 selective adenosine antagonist (KF17837) on the renal responses to CM. In sham-operated group, iohexol (2 ml/kg/min for 3 min) increased effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), whereas in renal insufficiency group (with subtotal nephrectomy), following transient increases in ERPF and GFR, CM markedly decreased ERPF (−46.5 ± 6.7%) and GFR (−51.2 ± 7.1%). In sham-operated group, theophylline and KF17837 markedly attenuated CM-induced increases in ERPF and GFR, while KW-3902 had no effects on CM-induced increases in ERPF or GFR. In renal insufficiency group, initial increases in ERPF and GFR were blunted by theophylline and KF17837. In contrast, the subsequent decreases in ERPF and GFR were attenuated by theophylline (%ΔERPF, −12.2 ± 3.2% vs. −46.6 ± 6.7%, $P < 0.01$; %ΔGFR, 4.3 ± 2.5% vs. −51.0 ± 7.1%, $P < 0.01$), and were completely prevented by KW-3902 (%ΔERPF, 10.8 ± 2.9%; %ΔGFR, 23.8 ± 4.4%), whereas KF17837 aggravated ERPF (−73.3 ± 5.3%) and GFR (−78.4 ± 5.3%). These data indicate that in normal renal function, iohexol elicits renal vasodilation by activating mainly the adenosine A2 receptors. In contrast, in impaired renal function, CM induces both A2 and A1 activation; the former is associated with the initial renal vasodilation, while the latter is responsible for the sustained aggravation of renal hemodynamics.

A recent development of non-ionic radiographic contrast media (CM) has contributed to the reduction in the incidence of CM-induced nephropathy. However, in patients with renal insufficiency, non-ionic CM fails to reduce the risk of CM-induced nephropathy [1–3]. Furthermore, clinical necessity for diagnostic procedures using CM has been increasing, especially in patients with cardiovascular diseases, whose renal function is frequently impaired. Despite such clinical demand, there have been no definitive measures to prevent CM-induced nephropathy.

Previous investigations suggested that renal hemodynamic changes constituted a determinant of CM-induced nephropathy. It has been demonstrated that following transient renal vasodilation, CM produces progressive vasoconstriction which consequently causes concomitant decreases in both renal blood flow and glomerular filtration rate (GFR) [4–6]. Numerous studies have been conducted to reveal the pathogenesis of CM-induced nephropathy. It has been shown that theophylline, an adenosine antagonist, prevents acute renal failure induced by CM in both animal models [6, 7] and humans [8]. These observations suggest that the renal protective effects of theophylline are mediated by the blockade of adenosine action. Theophylline, however, affects several aspects of vasomotor mechanisms since this agent is a relatively non-selective adenosine antagonist, and is also reported to inhibit phosphodiesterase [9]. Thus, the precise mechanisms for theophylline prevention remain undetermined.

In the present study, we examined the effects of non-ionic CM on renal hemodynamics in dogs with and without pre-existing renal insufficiency. Furthermore, the effects of a non-selective adenosine antagonist, theophylline, a newly developed selective adenosine A1 receptor antagonist, 8-(noradamantan-3-yl)-1,3-dipropylxanthine (KW-3902) [10–13], and a selective adenosine A2 receptor antagonist, (E)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF17837) [14–17], on CM-induced alterations in renal function were assessed to characterize the role of adenosine in mediating the CM-induced renal dysfunction.

Methods

Surgical preparation

Male beagles weighing 10 to 12 kg were divided into two groups: the control group, and the renal insufficiency group. All surgical procedures were performed under general anesthesia induced by pentobarbital sodium and maintained with halothane. Catheters (Tygon, U.S. Stoneware, Akron, OH, USA) were inserted into the right internal iliac artery and vein, externalized through the back between the scapulae, and secured. Subsequently, in the renal insufficiency group subtotal nephrectomy (left hemi-nephrectomy and ligation of two of three branches of the right renal artery) was performed [18, 19]; the control group had a sham operation. Upon completion of surgery, the dogs were placed in individual cages to allow free mobility. During the recovery period, all dogs were fed a diet (Oriental Yeast, Tokyo, Japan) containing 70 mmol sodium and 60 mmol potassium daily. Free access to tap water was permitted at all times. Serum creatinine concentration was measured every week. Only the dogs whose serum creatinine concentration exceeded 1.5 mg/dl had been included as renal insufficiency group in the study. All experiments were performed during 12 to 16 weeks after the operation.

All hemodynamic studies were performed in conscious dogs during fasting between 8 a.m. to 3 p.m. in a quiet room, and were

Received for publication July 28, 1995
and in revised form December 18, 1995
Accepted for publication December 19, 1995

begun after a 60-minute stabilization period. All procedures were conducted following the guidelines of the institutional Animal Care Committee.

Monitoring procedures

The arterial catheter was connected to a pressure transducer (model TP-400T, Nihon Kohden, Tokyo, Japan) for measurement of the mean arterial blood pressure (MAP) and heart rate (HR). The data for MAP and HR were analyzed on a Macintosh computer, using an analog-digital converter, the Macintosh Laboratory System (MacLab™; Analog Digital Instruments Pty., Ltd., Castle Hill, Australia), at a sample rate of 20 points/sec, as described previously [20–22].

Urine collection and administration of p-aminohippurate (PAH) and inulin

To assess the renal function including urine flow rate (UFR), PAH clearance and inulin clearance, 7 Fr. balloon catheters (Create Medic Co., Yokohama, Japan) were inserted into the bladder per urethra and urine was collected. Sixty minutes before starting control measurements, a bolus injection of 20% PAH (80 mg) and 25% inulin (500 mg) was carried out, and PAH (1.2 mg/min) and inulin (5 mg/min) at a rate of 0.5 ml/min were continuously infused to achieve stable blood levels.

Measurements of plasma renin activity (PRA), hormones, inulin and PAH

PRA was determined by radioimmunoassay (Dinabott Radioisotope, Tokyo, Japan) [23]. Plasma aldosterone concentration (PAC) was measured by radioimmunoassay (Daiichi Radioisotope, Tokyo, Japan) [24]. PAH and inulin were measured by colorimetry (Model 7010, Hitachi, Tokyo, Japan).

Study protocol

Experiment 1: Effects of CM on renal function. At 12 to 16 weeks after surgery, the baseline hemodynamics, including arterial blood pressure and HR, were measured in dogs with normal renal function (that is, sham operated; $N = 5$) and with renal insufficiency ($N = 5$) for several consecutive days. All dogs were prohibited from drinking and eating 24 hours before experiments. A bolus, followed by a continuous infusion of PAH and inulin were started, and after a one-hour stabilization period, control measurements were begun. After 60 minutes of control measurement, iohexol was administered intravenously for 3 minutes; 2 ml/kg/min for normal renal function, and 1, 2, and 3 ml/kg/min for renal insufficiency. Hemodynamic measurements were continued for two hours after the drug administration, and urine was collected every 30 minutes. Blood samples were obtained for measurement of the serum concentrations of creatinine, PAH, and inulin at the midpoint of each 30 minutes.

Experiment 2: Effects of adenosine antagonists in normal renal function. Effects of theophylline (200 μg/kg/min, i.v. for 150 min), KW-3902 (5 μg/kg/min, i.v. for 150 min) and KF17837 (10 μg/kg/min, i.v. for 150 min) on the renal responses to CM were investigated in dogs with normal renal function. Iohexol (2 ml/kg/min, i.v. for 3 min) was administered 30 minutes after infusion of theophylline ($N = 5$), KW-3902 ($N = 5$), KF17837 ($N = 5$) or vehicle (see below; $N = 15$). Hemodynamic measurements were continued for two hours after CM administration, and urine collection and blood sampling were carried out in the same manner as in the experiment 1. Theophylline was dissolved in saline, while KW-3902 and KF17837 were dissolved in dimethyl sulfoxide (DMSO) (100 μl DMSO for 1 mg KW-3902 and 2 mg KF17837). DMSO was used as vehicle for KW-3902 and KF17837.

Table 1. Changes in MAP, renal function, and hormones by subtotal nephrectomy

	Control	Renal insufficiency
MAP *mm Hg*	75 ± 9	81 ± 5
UFR *ml/hr*	10 ± 2	9 ± 1
GFR *ml/min*	26 ± 3	9 ± 1[a]
ERPF *ml/min*	108 ± 14	32 ± 3[a]
PRA *ng/ml/hr*	1.5 ± 0.3	1.8 ± 0.2
PAC *pg/ml*	62 ± 7	74 ± 5

Values are means ± SEM; $N = 5$. Abbreviations are: MAP, mean arterial pressure; UFR, urine flow rate; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; PRA, plasma renin activity; PAC, plasma aldosterone concentration.

[a] $P < 0.01$ vs. Control

Experiment 3: Effects of adenosine antagonists in renal insufficiency. Effects of theophylline (200 μg/kg/min, i.v. for 150 min), KW-3902 (5 μg/kg/min, i.v. for 150 min) and KF17837 (10 μg/kg/min, i.v. for 150 min) on the renal responses to CM were investigated in dogs with renal insufficiency. Iohexol (2 ml/kg/min, i.v. for 3 min) was administered 30 minutes after infusion of theophylline ($N = 5$), KW-3902 ($N = 5$), KF17837 ($N = 5$), or vehicle ($N = 15$). Hemodynamic measurements were continued for two hours after CM administration, and urine collection and blood sampling were carried out in the same manner as in the experiment 1.

Experiment 4: Effects of calcium antagonist (diltiazem) on CM-induced renal deterioration. Effects of diltiazem (5 μg/kg/min, i.v. for 150 min) on CM-induced renal deterioration were examined. Diltiazem ($N = 5$) or vehicle ($N = 5$) was injected 30 minutes before iohexol administration (2 ml/kg/min, i.v. for 3 min). Hemodynamic measurements were continued for two hours after CM administration. Diltiazem was dissolved in saline.

Materials

Iohexol (Omnipaque 300) was purchased from Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). KW-3902 and KF17837 were gifts from Kyowa Hakko Kogyo Co. Ltd. (Shizuoka, Japan). Diltiazem was purchased from Sigma Chemical (St. Louis, MO, USA).

Statistical procedures

Results are expressed as means ± SEM. Data were analyzed by one-way or two-way ANOVA as appropriate, followed by Scheffe's multiple comparison post hoc test. $P < 0.05$ was considered statistically significant.

Results

Effects of subtotal nephrectomy on hemodynamics and hormones

The data of MAP, renal function and hormones in control group and renal insufficiency group are shown in Table 1. There were no significant differences in MAP or UFR between two groups. Both ERPF (clearance of PAH) and GFR (clearance of

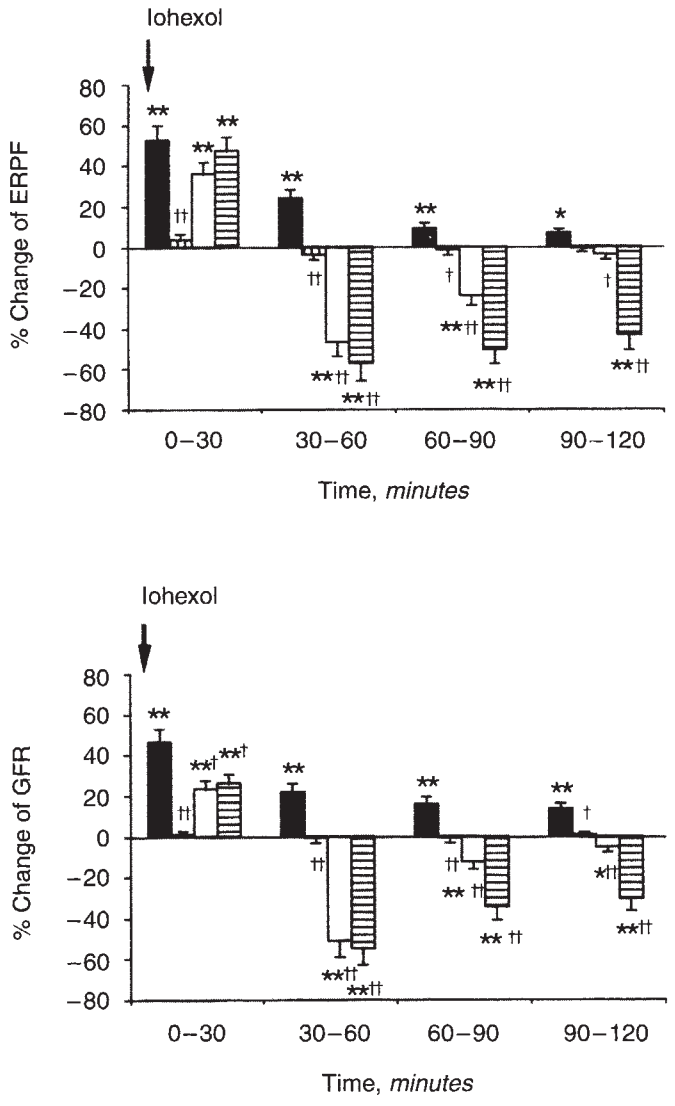

Fig. 1. *Effects of iohexol on effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) in dogs with and without renal insufficiency.* Values are means ± SEM. Symbols are: (■) Control group, iohexol 2 ml/kg/min; (▥) Renal insufficiency group, iohexol 1 ml/kg/min; (□) iohexol 2 ml/kg/min; (▤) iohexol 3 ml/kg/min. $*P < 0.05$, $**P < 0.01$ vs. baseline, $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ vs. normal renal function.

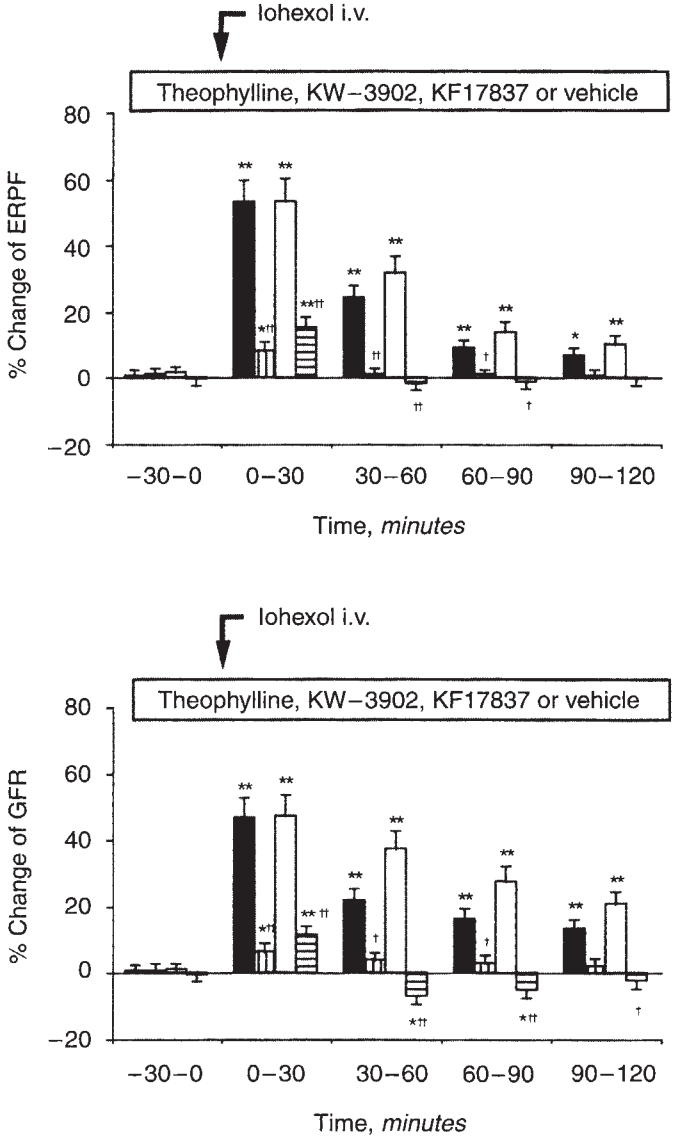

Fig. 2. *Effects of theophylline (200 μg/kg/min i.v.), KW-3902 (5 μg/kg/min i.v.) and KF17837 (10 μg/kg/min i.v.) on renal responses to iohexol (2 ml/kg/min i.v. for 3 min) in dogs with normal renal function.* Values are means ± SEM. Symbols are: (■) CM + vehicle; (▥) CM + theophylline; (□) CM + KW-3902; (▤) CM + KF17837. $*P < 0.05$, $**P < 0.01$ vs. baseline, $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ vs. vehicle.

inulin) were markedly decreased in dogs with renal insufficiency. No differences were noted in PRA or PAC between two groups.

Effects of CM on renal function

In dogs with normal renal function (control), administration of iohexol (2 ml/kg/min for 3 min) markedly increased both ERPF (52.8 ± 7.3%) and GFR (46.8 ± 6.7%) during 0 to 30 minutes (Fig. 1). Subsequently, they gradually decreased, but remained elevated throughout the experimental periods observed.

In dogs with renal insufficiency, a low dose of iohexol (1 ml/kg/min) did not alter ERPF or GFR. A medium dose of iohexol (2 ml/kg/min) elicited transient increments in ERPF (36.2 ± 5.6%) and GFR (23.2 ± 4.3%) during 0 to 30 minutes after injection, followed by significant reductions in ERPF and GFR during the subsequent 30 to 90 min (ERPF: 30 to 60 min, −46.5 ± 6.7%; 60 to 90 min, −24.3 ± 24.4%; GFR: 30 to 60 min, −51.2 ± 7.1%; 60 to 90 min, −12.8 ± 3.3%). A high dose of iohexol (3 ml/kg/min) decreased ERPF and GFR markedly, and the reduced renal function persisted throughout the experiment.

Effects of adenosine antagonists in normal renal function

In dogs with normal renal function, the administration of theophylline, KW-3902 or KF17837 alone did not alter basal MAP, HR, ERPF or GFR (Fig. 2, Tables 2 and 3).

We used DMSO as solvent for KW-3902 and KF17837, and saline for theophylline and diltiazem. Since CM-induced renal hemodynamic changes in the presence of each vehicle did not

Table 2. Effects of theophylline, KW-3902, KF17837 or diltiazem on MAP and HR

Time after administration	*N*	Baseline	45 min	75 min
Normal renal function group				
MAP *mm Hg*				
theophylline	5	78 ± 6	86 ± 5	84 ± 3
KW-3902	5	74 ± 8	72 ± 5	71 ± 2
KF17837	5	73 ± 5	77 ± 2	79 ± 4
HR *beats/min*				
theophylline	5	72 ± 6	80 ± 3	79 ± 5
KW-3902	5	77 ± 8	74 ± 5	75 ± 4
KF17837	5	75 ± 5	73 ± 2	74 ± 3
Renal insufficiency group				
MAP *mm Hg*				
theophylline	5	79 ± 4	86 ± 5	85 ± 4
KW-3902	5	81 ± 6	83 ± 3	81 ± 2
KF17837	5	83 ± 5	91 ± 4	92 ± 3
diltiazem	5	81 ± 6	83 ± 3	80 ± 5
HR *beats/min*				
theophylline	5	70 ± 4	77 ± 4	79 ± 3
KW-3902	5	72 ± 5	70 ± 3	71 ± 4
KF17837	5	74 ± 3	76 ± 2	73 ± 4
diltiazem	5	72 ± 5	62 ± 2[a]	58 ± 2[a]

Values are means ± SEM. Abbreviations are: MAP, mean arterial pressure; HR, heart rate, theophylline 200 μg/kg/min i.v.; KW-3902 5 μg/kg/min i.v.; KF17837 10 μg/kg/min i.v.; diltiazem 5 μg/kg/min i.v.
[a] $P < 0.05$ vs. baseline

Table 3. Effects of theophylline, KW-3902, KF17837 or diltiazem on ERPF and GFR

Time after administration	*N*	Baseline	30–60 min	60–90 min
Normal renal function group				
ERPF *ml/min*				
theophylline	5	110 ± 6	112 ± 5	114 ± 7
KW-3902	5	107 ± 8	110 ± 5	111 ± 6
KF17837	5	107 ± 5	105 ± 7	103 ± 8
GFR *ml/min*				
theophylline	5	32 ± 6	33 ± 4	34 ± 5
KW-3902	5	33 ± 8	35 ± 7	36 ± 7
KF17837	5	31 ± 5	30 ± 3	28 ± 6
Renal insufficiency group				
ERPF *ml/min*				
theophylline	5	34 ± 4	36 ± 3	35 ± 4
KW-3902	5	32 ± 3	33 ± 5	33 ± 2
KF17837	5	30 ± 5	31 ± 4	30 ± 3
diltiazem	5	32 ± 3	35 ± 5	34 ± 5
GFR *ml/min*				
theophylline	5	10 ± 2	10 ± 2	9 ± 2
KW-3902	5	9 ± 1	9 ± 2	10 ± 2
KF17837	5	9 ± 1	9 ± 2	8 ± 2
diltiazem	5	9 ± 1	10 ± 1	10 ± 1

Values are means ± SEM. Abbreviations are: ERPF, effective renal plasma flow; GFR, glomerular filtration rate; theophylline 200 μg/kg/min i.v.; KW-3902 5 μg/kg/min i.v.; KF17837 10 μg/kg/min i.v.; diltiazem 5 μg/kg/min i.v.

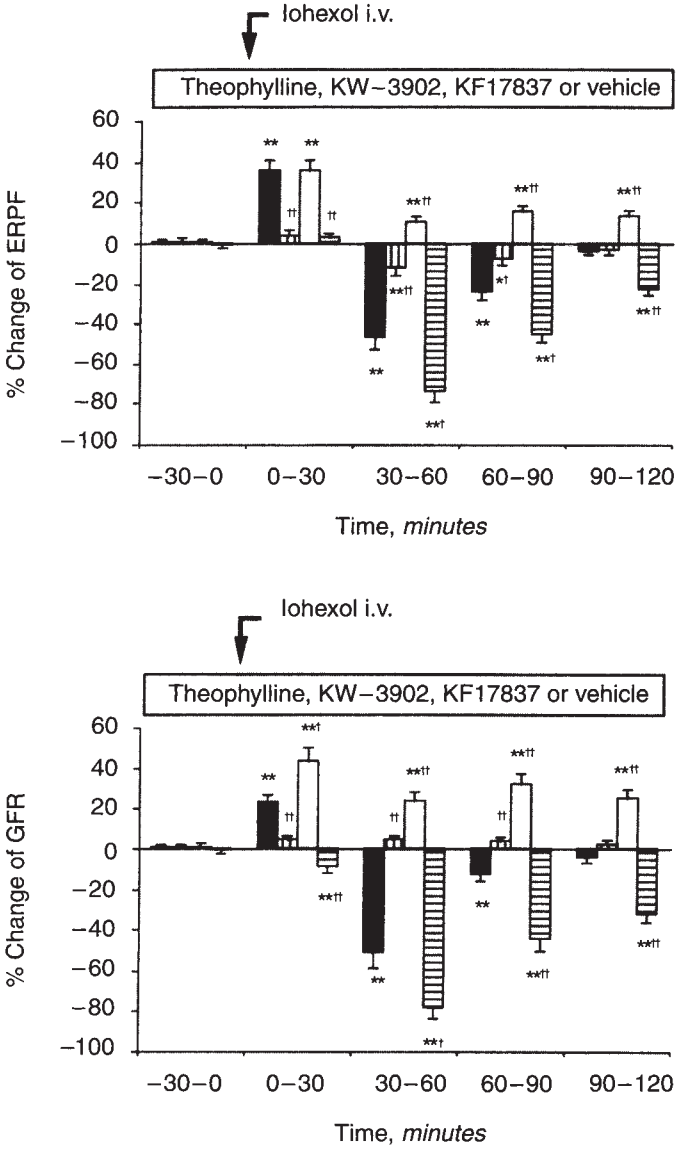

Fig. 3. *Effects of theophylline (200 μg/kg/min i.v.), KW-3902 (5 μg/kg/min i.v.) and KF17837 (10 μg/kg/min i.v.) on renal responses to iohexol (2 ml/kg/min i.v. for 3 min) in dogs with renal insufficiency.* Values are means ± SEM. Symbols are: (■) CM + vehicle; (▥) CM + theophylline; (□) CM + KW-3902; (▤) CM + KF17837. *$P < 0.05$, **$P < 0.01$ vs. baseline, †$P < 0.05$, ††$P < 0.01$ vs. vehicle.

differ, the results obtained from each vehicle study were incorporated into a single set of data and were depicted as "CM + vehicle" in Figures 2 to 4.

Theophylline markedly altered the CM-induced responses of ERPF and GFR. Thus, the CM-induced increases in ERPF were prominently diminished (0 to 30 min, 8.0 ± 2.8%; 30 to 60 min, 1.0 ± 1.6%; 60 to 90 min, 0.8 ± 1.4%), as compared with those in the absence of theophylline (0 to 30 min, 53.1 ± 7.3%, $P < 0.01$; 30 to 60 min, 24.0 ± 4.4%, $P < 0.01$; 60 to 90 min, 9.0 ± 2.9%, $P < 0.05$). Similarly, theophylline prominently suppressed the iohexol-induced increments in GFR (0 to 30 min, 6.3 ± 2.6% vs. 46.7 ± 6.7%, $P < 0.01$; 30 to 60 min, 3.9 ± 2.4% vs. 21.9 ± 4.2%, $P < 0.05$; 60 to 90 min, 2.9 ± 2.3% vs. 16.0 ± 3.6%, $P < 0.05$, for theophylline and vehicle, respectively).

The pretreatment with KW-3902 failed to alter the iohexol-induced responses of ERPF throughout the experimental periods. Similarly, KW-3902 had no effects on the increments in GFR induced by iohexol.

In contrast to the effects of KW-3902, the pretreatment with KF17837 prominently blunted the CM-induced increases in both ERPF and GFR. Thus, the iohexol-induced initial (that is, 0 to 30

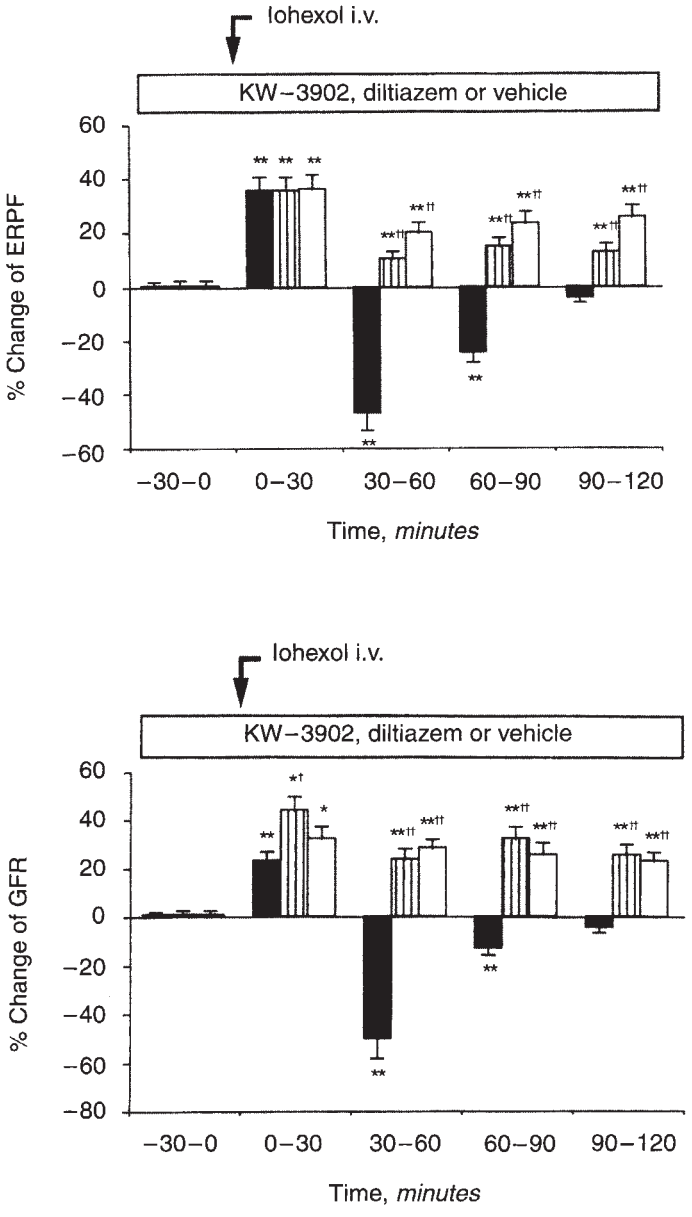

Fig. 4. *Effects of KW-3902 (5 μg/kg/min i.v.) and diltiazem (5 μg/kg/min i.v.) on declines in effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) induced by iohexol (2 ml/kg/min i.v. for 3 min) in dogs with renal insufficiency.* Values are means ± SEM. Symbols are: (■) CM + vehicle; (▥) CM + KW-3902; (□) CM + diltiazem. *$P < 0.05$, **$P < 0.01$ vs. baseline, †$P < 0.05$, ††$P < 0.01$ vs. vehicle.

min) increases in ERPF and GFR were markedly diminished (14.8 ± 3.5% and 11.2 ± 3.1%, respectively), as compared with those in the absence of KF17837 (52.9 ± 7.3%, $P < 0.01$ and 46.5 ± 6.7%, $P < 0.01$, respectively). Furthermore, KF17837 prevented the subsequent iohexol-induced increments in ERPF (30 to 60 min, −1.7 ± 2.2%; 60 to 90 min, −1.2 ± 2.1%). In analogy, iohexol failed to increase GFR in the presence of KF17837 (30 to 60 min, −6.9 ± 2.7%; 60 to 90 min, −5.2 ± 2.5%; 90 to 120 min, −2.4 ± 2.2%).

Effects of adenosine antagonists in renal insufficiency

In dogs with renal insufficiency, the administration of theophylline, KW-3902 or KF17837 alone had no effects on MAP, HR, ERPF or GFR (Fig. 3, Tables 2 and 3).

The pretreatment with theophylline suppressed both the initial increments and the subsequent decrements in ERPF. Thus, in the presence of theophylline, iohexol failed to alter ERPF during 0 to 30 minutes (4.1 ± 2.4%), and the CM-induced reductions in ERPF were attenuated during 30 to 90 minute (30 to 60 min, −12.2 ± 3.2%; 60 to 90 min, −7.6 ± 2.8%), as compared with marked declines in the absence of theophylline (30 to 60 min, −46.6 ± 6.7%, $P < 0.01$; 60 to 90 min, −24.5 ± 4.4%, $P < 0.05$). Similarly, theophylline attenuated the iohexol-induced initial increments in GFR observed during 0 to 30 minutes (4.6 ± 2.5% vs. 23.2 ± 4.3%, $P < 0.01$). Furthermore, reductions in GFR were completely abolished by theophylline (30 to 60 min, 4.3 ± 2.5% vs. −51.0 ± 7.1%, $P < 0.01$; 60 to 90 min, 3.6 ± 2.4% vs. −12.9 ± 3.3%, $P < 0.01$; for theophylline and vehicle, respectively).

KW-3902 not only prevented CM-induced renal deterioration, but actually maintained ERPF and GFR above baseline levels. Thus, ERPF increased during 0 to 30 minutes (35.8 ± 5.6%), and the increases in ERPF persisted throughout the experimental periods (30 to 60 min, 10.8 ± 2.9%; 60 to 90 min, 15.4 ± 3.2%; 90 to 120 min, 13.2 ± 3.2%), as compared with marked declines in the absence of KW-3902 (30 to 60 min, −46.5 ± 6.7%, $P < 0.01$; 60 to 90 min, −24.3 ± 4.4%, $P < 0.01$; 90 to 120 min, −3.8 ± 2.4%, $P < 0.01$). Similarly, KW-3902 enhanced the iohexol-induced initial increments in GFR during 0 to 30 minutes (43.8 ± 6.4% vs. 23.0 ± 4.3%, $P < 0.05$). Furthermore, in contrast to marked reductions in GFR in the absence of KW-3902 (30 to 60 min, −50.8 ± 7.1%; 60 to 90 min, −12.9 ± 3.3%; 90 to 120 min, −4.8 ± 2.5%), GFR remained elevated throughout the experimental periods (30 to 60 min, 23.8 ± 4.4%; 60 to 90 min, 32.0 ± 4.2%; 90 to 120 min, 25.6 ± 4.6%).

KF17837 not only blunted the CM-induced rise in renal function but also aggravated the CM-induced renal dysfunction. Thus, KF17837 abolished the CM-induced initial increment in ERPF (that is, 3.2 ± 1.7% vs. 35.8 ± 5.6%, $P < 0.01$). Furthermore, in the presence of KF17837, the CM-induced reductions in ERPF were markedly enhanced (that is, 30 to 60 min, −73.3 ± 5.3%; 60 to 90 min, −44.7 ± 4.0%; 90 to 120 min, −22.5 ± 3.2%), as compared with those in the absence of KF17837 (30 to 60 min, −46.5 ± 6.7%, $P < 0.05$; 60 to 90 min, −24.3 ± 4.4%, $P < 0.05$; 90 to 120 min, −3.8 ± 2.4%, $P < 0.01$). In analogy, KF17837 abolished the iohexol-induced initial increments in GFR during 0 to 30 min (−9.1 ± 2.9% vs. 23.0 ± 4.3%, $P < 0.01$). Furthermore, KF17837 aggravated the CM-induced declines in GFR (30 to 60 min, −78.4 ± 5.3% vs. −50.8 ± 7.1%, $P < 0.05$; 60 to 90 min, −44.5 ± 5.5% vs. −12.9 ± 3.3%, $P < 0.01$; 90 to 120 min, −32.0 ± 4.2% vs. −4.8 ± 2.5%, $P < 0.01$).

Effects of calcium antagonist (diltiazem) on CM-induced renal deterioration

The effects of diltiazem on CM-induced renal deterioration were assessed in dogs with renal insufficiency, and were compared with those of KW-3902 (Fig. 4).

The dose of diltiazem used in the experiment did not alter basal MAP, ERPF or GFR, while modestly decreased HR (Tables 2 and 3).

Diltiazem completely prevented the CM-induced reductions in ERPF and GFR. Thus, ERPF was increased during 0 to 30 minutes after CM administration (36.2 ± 5.6%), and the increases in ERPF persisted throughout the experimental periods (30 to 60 min, 20.3 ± 4.0%; 60 to 90 min, 23.6 ± 4.4%, 90 to 120 min, 25.7 ± 4.6%); the increments in ERPF were nearly identical in magnitude with those in the presence of KW-3902. Similarly, in the presence of diltiazem, the CM-induced increase in GFR (that is, 32.1 ± 5.1% at 0 to 30 min) was maintained above pre-CM level throughout the study (30 to 60 min, 28.1 ± 4.3%; 60 to 90 min, 25.9 ± 4.7%; 90 to 120 min, 22.7 ± 4.2%).

Discussion

In the present study, we have demonstrated that in dogs with normal renal function, a non-ionic CM, iohexol, causes sustained increases ERPF and GFR. In striking contrast, in dogs with pre-existing renal insufficiency, following transient increments in ERPF and GFR, CM deteriorates prominently the renal hemodynamics. Furthermore, administration of a non-selective adenosine antagonist, theophylline, and a selective adenosine A2 receptor antagonist, KF17837, markedly attenuate the CM-induced increases in ERPF and GFR both in normal and in impaired renal function. Additionally, in dogs with renal insufficiency, the CM-induced renal deterioration was prevented by theophylline, a selective A1 receptor antagonist (KW-3902), and a calcium antagonist (diltiazem). Thus, the present study demonstrates that adenosine plays an important role in CM-induced deterioration of renal insufficiency. Furthermore, multiple vasoregulatory mechanisms contribute to the CM-induced alterations in renal hemodynamics; the CM-induced vasoconstriction is mediated by adenosine A1 receptors and the subsequent calcium influx through voltage-operated calcium channels, whereas adenosine A2 receptors are responsible for the CM-induced renal vasodilation.

Contribution of non-ionic CM to reducing the incidence of acute renal failure remains to be determined. Initially, the introduction of non-ionic CM was expected to reduce the incidence of CM-induced nephropathy, based on the results observed in animals with normal renal function [25, 26]. The subsequent clinical investigations, however, failed to demonstrate a less nephrotoxicity of non-ionic CM in patients with renal insufficiency [1–3]. It appears, therefore, that the risk of non-ionic CM-induced nephropathy depends on the pre-existing renal insufficiency.

To confirm whether pre-existing renal insufficiency contributes to the development of non-ionic CM-induced nephropathy, we examined the effects of non-ionic CM on renal function in dogs with and without renal insufficiency. Thus, the present study has demonstrated that after only transient increases in ERPF and GFR, iohexol produces marked reductions in ERPF and GFR in renal insufficiency dogs. In contrast, in dogs with normal renal function, iohexol elicited prominent increases in ERPF and GFR, and they remained elevated throughout the study. Sustained deterioration in renal hemodynamics, observed only in the setting of renal insufficiency, could therefore constitute a critical determinant of the development of CM-induced ARF.

Although the precise mechanisms of CM-induced deterioration of renal function remain undetermined, several investigations have suggested that adenosine mediates CM-induced renal vasoconstriction [6, 7]. Thus, theophylline, a non-selective adenosine antagonist, markedly inhibited the CM-induced renal vasoconstriction [6]. Furthermore, the administration of theophylline prevented the renal deterioration in patients with renal failure [8]. In the present study, we have demonstrated that both non-selective (that is, theophylline) and A1-selective adenosine receptor antagonists (KW-3902) markedly prevent CM-induced deterioration in renal function in dogs with renal insufficiency. Additionally, infusion of adenosine is reported to elicit marked decreases in renal blood flow [27–29]. In concert, these observations strongly suggest that intrarenally-formed adenosine is involved in the development of CM-induced renal deterioration, which may lead to acute renal failure.

Several lines of recent investigations have clarified that adenosine acts at least via two different receptor subtypes. Of these, adenosine A1 receptors are associated with vasoconstriction. With respect to renal hemodynamics, adenosine A1 receptor agonists [30, 31] are reported to cause renal vasoconstriction. Thus, in the setting under which adenosine is responsible for the renal deterioration, the blockade of adenosine A1 receptors would ameliorate the renal dysfunction. In the present study, we have demonstrated that a novel, potent and selective adenosine A1 receptor antagonist, KW-3902 [10–13], completely prevents the CM-induced aggravation of renal function in dogs with renal insufficiency; rather, CM-induced increments in ERPF and GFR were maintained elevated throughout the experimental periods in the presence of KW-3902 (Fig. 3). In contrast, KW-3902 alone had no effects on renal hemodynamics (Table 3). Collectively, our present findings indicate that adenosine A1-related renal vasoconstriction is involved in the CM-induced deterioration of renal insufficiency. We propose that the detrimental effects of CM are mediated by adenosine A1 receptors.

The present study has demonstrated that theophylline not only prevents the CM-induced deterioration in renal hemodynamics in renal insufficiency, but also attenuates the increases in ERPF and GFR in both normal and impaired renal function. The latter observations indicate that adenosine participates in the CM-induced increases in renal hemodynamics. In this regard, adenosine causes vasodilation mediated by adenosine A2 receptor subtypes in most vascular beds [32–34]. In the renal vasculature, it has been demonstrated that adenosine A2 agonists elicit renal vasodilation [35, 36]. The present study shows that KF17837, a selective adenosine A2 antagonist, attenuates the CM-induced increments in ERPF and GFR in both normal and impaired renal function, and aggravates the CM-induced renal deterioration in renal insufficiency (Fig. 3). In concert, these observations indicate that adenosine A2 receptor-mediated vasodilation contributes to the CM-induced increases in renal hemodynamics in normal renal function, and also counters the adenosine A1-mediated renal aggravation in impaired renal function.

Of note, in dogs with normal renal function, the blockade of adenosine A2 receptors by KF17837 almost completely abolished the CM-induced responses, whereas blocking A1 receptors by KW-3902 had only modest effects on renal hemodynamics. These findings suggest that adenosine A2 receptor-mediated renal vascular responses constitute a major determinant of CM-induced alterations in renal hemodynamics under normal renal function. In striking contrast, in dogs with renal insufficiency, adenosine A1 blockade converted the CM-induced decreases to the increases in both ERPF and GFR, indicating a predominant role of adenosine A1 receptors in mediating the CM-induced renal responses. It appears, therefore, that the relative contribution of adenosine A1 and A2 receptors to CM-induced renal responses may be altered

in renal insufficiency, and the exaggerated A1 subtype-linked renal responses could account for the CM-induced exaggerated deterioration in renal hemodynamics in impaired renal function. The preferential activation of adenosine A1 receptors and the subsequent renal vasoconstriction by CM in renal insufficiency might indicate that selective adenosine A1 antagonists, devoid of antagonism of vasodilatory adenosine A1 receptors, are preferable for the protection of renal function from CM-induced renal deterioration.

It has been suggested that calcium ion plays an important role in CM-induced renal dysfunction and in mediating the adenosine-induced renal vasoconstriction. The present study indicates that adenosine A1 receptors are involved in the CM-induced renal hemodynamic changes. Furthermore, activation of adenosine A1 receptors is associated with a decrease in cAMP, which could subsequently alter the activity of voltage-operated calcium channels [37–39]. Thus, adenosine-induced renal vasoconstriction is reported to be inhibited by blocking voltage-operated calcium channels [37, 39]. In concert, these observations allow one to speculate that CM-induced renal vasoconstriction is linked to voltage-operated calcium channels. Indeed, our present finding and the reports from other laboratories [6, 40] have demonstrated that CM-induced renal vasoconstriction is blocked by voltage-operated calcium channels. CM-induced renal vasoconstriction is, therefore, mediated by a central common vasoconstrictor mechanism, that is, calcium entry through voltage-operated calcium channels.

In conclusion, we have demonstrated that in dogs with normal renal function, non-ionic CM, iohexol, causes prominent vasodilation, mediated by adenosine A2 receptors. In contrast, in dogs with renal insufficiency, the CM induces a transient renal vasodilation, followed by a sustained and profound vasoconstriction. The initial vasodilation is mediated by adenosine A2, and the sustained vasoconstriction by adenosine A1 receptor-linked responses. Furthermore, a calcium antagonist, diltiazem, inhibits the CM-induced aggravation in renal hemodynamics, suggesting that voltage-operated calcium channels mediate the CM-induced renal dysfunction as a common vasoconstrictor pathway. The present study indicates a major role for adenosine in the development of non-ionic CM-induced renal deterioration. The alterations in relative contribution of adenosine A1 and A2 receptor subtypes to CM-induced renal responses may be responsible for the divergent renal hemodynamic responses observed in normal and impaired renal function. The predominant adenosine A1 activation by CM in renal insufficiency could increase the susceptibility of the kidney with impaired function to contrast media.

Acknowledgments

We thank Kyowa Hakko Kogyo Co. Ltd. for providing adenosine A1 receptor antagonist, KW-3902, and adenosine A2 receptor antagonist, KF17837. We are grateful to K. Suzuki for excellent care for the animals.

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